J Infect Chemother (2001) 7:69–80

REVIEW ARTICLE

Dennis L. Stevens

Invasive streptococcal infections

Received: February 14, 2001

Introduction

In the 15 years since the first description of the streptococcal toxic shock syndrome (StrepTSS), reports have documented the presence of these invasive infections in all corners of the world, in all races, sexes, and age groups. The strains responsible for these infections have been similar, suggesting a global spread of infection. As might be expected, identical strains have also accounted for less serious infections, such as cellulitis, pharyngitis, and even asymptomatic carriage. Thus, host factors and co-morbid conditions must account for the different diseases caused by identical strains.

The most prevalent M-type associated with StrepTSS has been, and continues to be, M-type 1. Interestingly, M-type 3 strains have always been in second place and, together, M-types 1 and 3 account for approximately 50% of cases. Recent studies in the Netherlands found M-1 and M-3 strains each accounted for 25% of cases of invasive group A streptococcal infection.[1] In some geographical areas, M-1 strains have accounted for 70% of invasive infections. In contrast, in the epidemic that occurred in Wannamingo, Minnesota, 100% of the strains were M-3.[2] Other strains less commonly associated with StrepTSS include M-types 4, 6, 11, 18, 28, and non-typeable strains (reviewed in[3]). In most geographical settings, a mixture of strains is responsible for sporadic cases.

As remarkable as the emergence of invasive group A streptococcal infections has been, the persistence of such infections worldwide is equally unusual. The increased incidence of severe group A streptococcal infections during the later part of the 1990s suggests that the prevalent strains, largely M-1 and M-3 strains, have the ability to persist in the human environment for indefinite periods. In fact, these strains have been widely distributed for decades as causes of simple pharyngitis and asymptomatic carriage. Thus, much of the world's population has been exposed to these strains, likely on numerous occasions. Despite this exposure and the type-specific immunity that should be generated, the *increasing* prevalence of M-1 strains in certain regions is troubling. For example, in Norway[4] and Sweden,[5] the recent prevalence of M-1 strains among invasive streptococcal infections, as well as pharyngeal isolates, has increased to greater than 70%. From the theoretical perspective, the high prevalence and persistence of such strains could be due to an absence of protective immunity in a significant portion of the human population, or could be due to changes in protective epitopes in the organism analogous to antigenic drift among influenza viruses. There is some data supporting the latter hypothesis. Although restriction fragment length polymorphism (RFLP) patterns of M-type 1 strains suggest that these strains have remained identical over a 10-year period, biophysical studies imply that this may not be the case. For example, de Malmanche and Martin,[6] using functional studies of opsonophagocytosis, demonstrated that immunity may only be strain, and not M-type, specific. In some of these strains, marked differences in the amino acid sequence of M-1 strains was apparent, despite the fact that these strains were serologically typed as M-1. In addition, Villasenor[7] has demonstrated that, among 75 strains of M-1 group A streptococci, susceptibility to opsonophagocytosis in the presence of a single polyclonal anti-M-1 sera followed a normal distribution curve, and that some M-type 1 strains were no better opsonized than heterologous, non-M-1 strains. Representative strains, selected on the basis of their susceptibility to phagocytosis, had differences in the sequence of M-protein in both the hypervariable region and the A-repeat regions of the molecule. Clearly, these changes did not affect the structure enough to alter the serologic M-type, but were sufficient to affect opsonization with convalescent sera. Thus, subtle differences in the surface structure of M-1 protein render some strains resistant to immune sera, suggesting that antigenic drift could

D.L. Stevens
Infectious Diseases Section, Veterans Affairs Medical Center, 500 West Fort St. (Bldg 45), Boise, ID 83702, USA
Tel. +1-208-422-1599; Fax +1-208-422-1365
e-mail: dlsteven@mindspring.com
University of Washington School of Medicine, Seattle, WA, USA

account for the high prevalence and persistence of M-1 strains for the past 15 years.

The increased incidence of invasive group A streptococcal infections also suggests a change in the virulence of the organism. M-protein, streptolysin O (SLO), and streptococcal pyrogenic exotoxins A and B (SPEA, SPEB) have been implicated as important virulence factors. However, none of these has been associated with all invasive cases. For example, although M-types 1 and 3 have been most common, M-types 12, 28, and non-typeable strains have been also associated with invasive streptococcal infection.[8] Similarly, in the United States, M-type 1 strains containing the gene for SPEA have been commonly associated with StrepTSS; however, only about 50% of these strains produce SPEA.[9] Thus, no single surface component or extracellular toxin has, thus far, been identified that correlates with the recent increase in virulence in group A streptococcus. In 1997, we reported a strong association between isolates from invasive disease, regardless of M-type, and the production of nicotinamide adenine dinucleotide glycohydrolase (NADase).[10] In contrast, isolates that are most commonly associated with rheumatic fever, i.e., M-types 5 and 18, did not produce NADase.[10] Throat isolates associated with uncomplicated symptomatic pharyngitis were most commonly M-types 1, 12, and 4,[8] and 100% of these isolates from the early 1980s to the present time were NADase producers.[10] This is not surprising, because the source of bacteria in invasive streptococcal infections is frequently the throat.

It is of special interest that M-type 1 strains isolated from recent (circa 1980) invasive cases were NADase producers,[10] because, historically, M-type 1 group A streptococci have been characteristically non-NADase producers.[11,12] This observed shift in NADase production has also been reported by Karasawa et al.[13] among M-type 1 isolates in Japan, and most recently by Ajdic et al.,[14] at the University of Oklahoma, where the group A streptococcal genome has been elucidated. Our genetic analysis demonstrated that all strains of group A streptococci, regardless of their ability to produce NADase, possess the NADase gene, *nga*, and that all isolates examined were more than 96% identical in *nga* and upstream regulatory sequences.[15] Further, because NADase-negative strains did not produce any immunoreactive protein or peptide fragment, we concluded that additional regulatory element(s) control NADase production.[15] Taken together, these findings suggest that, in the early 1980s, serotype M-1 underwent a stable genetic change, resulting in the ability to produce NADase. Further, acquisition of this trait occurred in temporal association with the emergence of M-type 1 group A streptococcus as the predominant serotype associated with severe invasive infections.[8,16,17] NADase's role in pathogenesis is currently under study.

Bacteremia

Until recently, group A streptococcal bacteremia occurred most commonly in the very young and the elderly.[17] Among children, predisposing factors included burns, varicella, malignant neoplasm, immunosuppression, and age less than 2 years.[17] Patients with septic scarlet fever had complications, such as extension of infection into the sinuses, peritonsillar tissue, or mastoids,[17] yet, bacteremia was uncommon.[18] Among the children with varicella studied by Bullowa and Wishik[18] in 1935, group A streptococcal bacteremia occurred in only approximately 0.5% of patients. In contrast, recently, Doctor et al.[19] found a 50% incidence of group A streptococcal bacteremia in patients hospitalized with varicella.

In elderly patients, the source of group A streptococcal infection is invariably the skin and is associated with cellulitis or erysipelas.[17] Group A streptococcal sepsis in the elderly (mean age, 50–60 years) has also been associated with diabetes, peripheral vascular disease, malignancy, and corticosteroid use.[17] Not surprisingly, mortalities of 35%–80% have been described in this patient population.[17]

In the United States and Europe during the 1850s, the occurrence of group A streptococcal bacteremia was rare in individuals 14–40 years of age,[17] and was primarily associated with puerperal sepsis. Recently however, intravenous drug abuse has emerged as a leading cause of group A streptococcal bacteremia in this age group.[17] Martin and Hoiby[4] have comprehensively demonstrated that the prevalence of group A streptococcal bacteremia in Norway in the late 1980s increased in all age groups, but the greatest increase (600%–800%) was in adolescents and young adults. Thus, the demographics of invasive group A streptococcal infections, with bacteremia as a marker, have changed dramatically in the past two decades.

Lymphangitis

Lymphangitis occurs most commonly during the course of an acute infection of the skin, usually cellulitis. It is most common following hemolytic streptococcal infections caused by groups A, C, and G streptococci. Red streaks beginning at the margin of skin infection and extending proximally are easily recognized and may extend several inches per hour. Thus, lymphangitis associated with a soft-tissue infection is likely a marker for a more aggressive strain of streptococci, and its recognition should prompt aggressive antibiotic therapy and close observation for evidence of bacteremia.

Invasive infections of the respiratory tract

Peritonsillar abscess, retropharyngeal abscess, and cervical lymphadenitis

Group A streptococci are normal inhabitants of the nasopharynx in 15%–20% of children and, understandably, streptococcal pharyngitis and tonsillitis are among the most common childhood afflictions. While tonsillitis is generally

attributed to streptococci, other organisms, including anaerobic and aerobic mouth flora, commonly contribute. Still, the group A streptococcus was found alone or in combination with other organisms in 100% of tonsils removed from 45 different children.[20] In some cases, extension of infection from the tonsil to adjacent cervical nodes, the peritonsillar soft tissues of the neck, or the retropharyngeal space results in necrotizing infections or abscess, which require surgical drainage or debridement. Peritonsillar abscesses are readily cured with penicillin plus needle aspiration.[21] Like streptococcal pharyngitis, retropharyngeal abscess is most common in children less than 6 years to age. These infections may be caused by group A streptococcus, *Bacteroides*, *Peptostreptococcus*, or *Staphylococcus aureus*. Aggressive imaging of the neck, with computerized axial tomography (CT) or magnetic resonance imaging (MRI), and emergent surgical consultation, are critically important because of the likelihood of upper airway obstruction, bacteremia, and extension of infection along complex fascial planes.

Mediastinitis

Group A streptococcal infections can reach the mediastinum via lymphatics from head and neck infections or soft tissue infection of the thorax, from contiguous spread from pneumonia, empyema, or parapharyngeal infection, or hematogenously from bloodstream infection. Mediastinal infections caused by group A streptococcus have a high mortality caused, in part, by the potency of toxins produced, the difficulty in making an early diagnosis, and finally, the difficulty in adequately draining the site of infection because of the abundance of vital structures in this anatomical site.

Pneumonia and empyema

Although uncommon among community-acquired infections, pneumonia caused by group A streptococcus is unique for two reasons. First, empyema develops in nearly 40% of cases, and second, pleural fluid accumulates rapidly, and may be as much as several liters per day. In the past, the greatest challenges for physicians treating patients with this infection were adequate drainage of loculated empyema fluid and the management of restrictive lung disease that developed subsequent to fibrosis of pleural surfaces. Shock and organ failure were uncommon. In contrast, recent cases of group A streptococcal pneumonia and empyema have been associated with the StrepTSS (author's observations).

Puerperal/post-partum sepsis

Puerperal sepsis occurs during pregnancy or abortion, when group A streptococcus colonizing the patient invades the endometrium and surrounding structures, as well as the lymphatics and bloodstream. The result is acute endometritis and bacteremia complicated by pelvic thrombophlebitis and peritonitis. Puerperal sepsis reached epidemic proportions in Europe[22] and the United States[23] during the mid-1800s, largely as the result of contamination of women by attending physicians. A small number of cases attributed to group B streptococcus was reported during this time,[24] and this organism remains an uncommon cause of puerperal sepsis. From 1900 to 1970, improvements in infection control practices and the advent of antibiotics significantly reduced the incidence of this infection. In contrast, currently in the United States,[25,26] Europe,[27–29] and Japan,[30] there has been a re-emergence of group A streptococcal puerperal sepsis associated with StrepTSS. While most of these cases have been sporadic, secondary cases associated with rectal or vaginal carriage of group A streptococcus by health-care workers have been reported.[31] Clearly, if more than one case occurs in a health facility, searches for a common source infection should be carried out.

Necrotizing soft-tissue infections

Necrotizing fasciitis

Necrotizing fasciitis is a deep-seated infection of the subcutaneous tissue that results in progressive destruction of fascia and fat, but with sparing of the skin itself. Historically, Pfanner[32] is credited with the first description of what he called necrotizing erysipelas. Several years later, Meleney[33] described 20 patients with hemolytic streptococcal gangrene in China, and he argued that this entity was different from erysipelas and should have a different name. These cases were probably caused by group A streptococci as we now know them, although at that time they only characterized the organism as hemolytic streptococci. Subsequently, "necrotizing fasciitis" has become the term that is preferred, because bacteria other than streptococci, such as *Clostridium perfringens*, *Clostridium septicum*, *Staphylococcus aureus*, and mixed aerobic-anaerobic bacteria can produce a similar pathologic process. Meleney's descriptions of the progression are clinically useful and are summarized as follows:

Characteristically streptococcal gangrene begins at the site of trivial or inapparent trauma. Within 24 hours of the initial lesion, there is aggressive development of swelling, heat, erythema and tenderness with rapid spreading proximally and distally from the original focus. During the next 24–48 hours, the erythema darkens, changing from red to purple and then to blue, and blisters and bullae form which contain clear yellow fluid. On the fourth or fifth day, the purple areas become frankly gangrenous. From the seventh to tenth day, the line of demarcation becomes sharply defined and the dead skin begins to separate at the margins revealing extensive necrosis of the subcutaneous tissue. Up until this time, the patient continues to be febrile, prostration increases and the patient becomes more emaciated. In more severe cases, the process advances rapidly until several large areas of skin have become gangrenous, and the

intoxication renders the patient dull, unresponsive, mentally cloudy or even delirious. Subsequently, the patient may develop metastatic abscesses, bronchopneumonia or lung abscess. (Adapted from reference[33].)

In should be remembered that Meleney's description of hemolytic streptococcal gangrene was published in 1924 – well before the advent of antibiotics. In fact, he was the first to advocate aggressive "bear scratch" fasciotomy and debridement.[33] Using this approach, as well as Dakan's solution for irrigation, mortalities of as low as 20% were achieved.[33] In contrast, more modern series suggest that mortalities of 30%–60% are common, even with antibiotics and aggressive surgical debridement. In addition, the progression of modern group A streptococcal infections is much more rapid than Meleney described, and frequently patients may die within 48–96 h of the onset of symptoms.[16] Further, these necrotizing processes are more likely to destroy skin, subcutaneous fat, fascia, and underlying muscle. For example, in one study,[16] myonecrosis was present in about 50% of patients who had necrotizing fasciitis. These data suggest that modern strains are more virulent.

Streptococcal myositis/myonecrosis

Group A streptococcal myositis has been an extremely uncommon infection. Adams et al.[34] documented only 21 cases that had been reported from 1900 to 1985, and Svane[35] found only 4 cases among over 20 000 autopsies. In our series of 20 patients with invasive group A streptococcal infection,[16] 1 had myositis alone, 3 had myositis as well as necrotizing fasciitis, and 5 had necrotizing fasciitis alone. Recently, deep soft-tissue infections, such as necrotizing fasciitis and myositis, have also been described in reports from Norway,[4] Sweden,[36] and Canada.[37] Although group A streptococci can infect viable muscle via a direct penetrating injury or surgical incision, in approximately 50% of cases there is no history of such an event. These cases develop spontaneously, or secondary to very minor blunt trauma or muscle strain, and most patients deny antecedent symptomatic pharyngitis or tonsillitis.[4,16,34–36,38,39] Thus, translocation of group A streptococci likely occurs from the colonized pharynx to the site of muscle injury via hematogenous or lymphatic spread. Severe pain may be the only presenting symptom, and physical findings early may demonstrate only swelling and erythema, although patients may rapidly develop muscle compartment syndromes.[4,16,34–36,38–40] In most cases, a single muscle group may be involved; however, because patients are frequently bacteremic, multiple sites of myositis can occur.[16,34] Distinguishing streptococcal myositis from spontaneous gas gangrene caused by *Clostridium perfringens* or *Clostridium septicum*[41] may be difficult, although the presence of crepitus or gas in the tissue would favor clostridial infection.[39] Distinguishing necrotizing fasciitis from myositis is easily done anatomically from surgical exploration or incisional biopsy. In streptococcal cases, many patients may have evidence of both necrotizing fasciitis and myositis.[16,34] In published series, the case fatality rate for necrotizing fasciitis is between 20% and 50%, whereas group A streptococcal myositis has a fatality rate between 80% and 100%.[16,34–36,38,39,41] Aggressive surgical debridement is of extreme importance, because of the poor efficacy of penicillin that has been described in human cases,[16,34,35,38,39] as well as in experimental streptococcal models of myositis[42,43] (see section on antibiotic efficacy). Of note, the term "myositis" connotes "inflammation" of muscle; however, in most cases of streptococcal myositis, frank necrosis is present, with only a paucity of infiltrating inflammatory cells. Thus, a better term would be "myonecrosis".

Table 1. Types of invasive group A streptococcal infections

- Bacteremia
- Lymphangitis
- Invasive infections of the respiratory tract
  - Peri-pharyngeal abscesses
  - Mediastinitis
  - Pneumonia
  - Empyema
- Post-partum sepsis
- Necrotizing soft-tissue infections
  - Necrotizing fasciitis
  - Myositis/myonecrosis
- Streptococcal toxic shock syndrome

Table 2. Defining the streptococcal toxic shock syndrome: an acute, febrile illness that begins with a mild viral-like prodrome and involves minor soft-tissue infection that may progress to shock, multiorgan failure, and death

Symptoms
- Early symptoms are vague
  - Viral-like prodrome
  - Severe pain and erythema of an extremity
  - Mental confusion

Signs
- Hypotension, systolic
- Fever, temperature >38°C
- Soft-tissue swelling
- Tenderness
- Respiratory failure, rales, cyanosis, tachypnea

Laboratory features
- Hematologic
  - Marked left shift (myelocytes, metamyelocytes)
  - Decline in hematocrit
  - Thrombocytopenia
- Renal azotemia (2.5 × normal on admission) and hematuria
- Hypocalcemia
- Hypoalbuminemia
- Creatinine phosphokinase elevation
- Pulmonary abnormalities
  - Pulmonary infiltrate on chest X-ray film
  - Hypoxia

Streptococcal toxic shock syndrome (StrepTSS)

Epidemiology

Several population-based studies of StrepTSS have documented the annual incidence of 1–5 cases per 100 000 popu-

lation,[44] with most cases being sporadic in nature; however, larger epidemics of invasive group A streptococcal infections have also been described in some settings. In 1994, an epidemic of related invasive infections occurred in Wannamingo, Minnesota,[2] with an annualized prevalence of 24 cases per 100000 population. In Missoula, Montana in 1999, the incidence of invasive infections reached 30 cases per 100000 population. In addition to community-based infections, invasive group A streptococcal infections have also been described in hospitals, convalescent centers, and among hospital employees and family contacts of patients with invasive infections.[9,45,46] Some of these studies have documented the same M-type and identical RFLP patterns in strains from primary and index cases.[9,45–47] In addition, carriage of group A streptococcus by healthcare personnel has been associated with the spread of life-threatening group A streptococcal infections in the obstetrics/gynecology and ear-nose-throat wards of American hospitals.[31] Such infections have also originated in outpatient surgical settings and within the home environment.

It has been estimated that the risk of secondary cases may be approximately 200 times greater than the risk among the general population.[48] There is ample data from studies conducted over several decades that group A streptococcus is quickly and efficiently transmitted from index cases to susceptible individuals, and that transmission may result in colonization, pharyngitis, scarlet fever, rheumatic fever, or invasive group A streptococcal infections. The risk for secondary cases is likely related to close or intimate contact and crowding, as well as host factors such as: (1) active viral infections such as varicella or influenza; (2) recent surgical wounds and childbirth (author's unpublished observations); (3) absence of type specific opsonic antibody against the group A streptococcus causing the index case; and (4) absence of neutralizing antibody against pyrogenic exotoxin A or B.[1]

Acquisition of group A streptococcus

The portals of entry for streptococci are the vagina, pharynx, mucosa, and skin in 50% of cases.[16] Interestingly, a portal cannot be defined in the remaining 50%.[16] Rarely, patients with symptomatic pharyngitis develop StrepTSS. Surgical procedures, such as suction lipectomy, hysterectomy, vaginal delivery, bunionectomy, and bone pinning provide a portal of entry in some cases. Numerous cases have developed within 24–72 h of minor non-penetrating trauma resulting in hematoma, deep bruise to the calf, or even following muscle strain.[16] Virus infections such as varicella and influenza have provided portals in other cases.[16] In some cases, the use of nonsteroidal anti-inflammatory agents may have either masked the presenting symptoms or predisposed to more severe streptococcal infection and shock.[16] Most cases of StrepTSS occur sporadically, although outbreaks of severe group A streptococcal infections have been described in closed environments, such as nursing homes,[49,50] and hospital environments.[45,46]

Symptoms and signs

Twenty percent of patients have an influenza-like syndrome, characterized by fever, chills, myalgia, nausea, vomiting, and diarrhea.[16] Fever is the most common presenting sign, although hypothermia may be present in patients with shock.[16] Confusion is present in 55% of patients, and in some, coma or combativeness are manifest.[16] Pain – the most common initial symptom of StrepTSS – is abrupt in onset, severe,[16] and usually precedes tenderness or physical findings. The pain usually involves an extremity, but may also mimic peritonitis, pelvic inflammatory disease, pneumonia, acute myocardial infarction, or pericarditis.[16] Eighty percent of patients have clinical signs of soft-tissue infection, such as localized swelling and erythema, which progresses to necrotizing fasciitis or myositis in 70% of cases, requiring surgical debridement, fasciotomy, or amputation.[16] Of the 20% of cases without soft-tissue findings, a variety of clinical presentations were observed, including endophthalmitis, myositis, perihepatitis, peritonitis, myocarditis, and overwhelming sepsis.[16] A diffuse, scarlatina-like erythema is uncommon, occurring in only 10% of cases.

Laboratory evaluation of patients with StrepTSS

The serum creatinine phosphokinase (CPK) level is useful in detecting the presence of deeper soft-tissue infections, and when the level is elevated or rising, there is a good correlation with necrotizing fasciitis or myositis.[16] Although the initial laboratory studies may demonstrate only mild leukocytosis, the mean percentage of immature neutrophils (including band forms, metamyelocytes, and myelocytes) is striking, reaching 40%–50%.[16] The presence of hemoglobinuria and elevated serum creatinine values is evidence of renal involvement. It is important to note that renal impairment precedes hypotension in 40%–50% of cases.[16] Hypoalbuminemia and hypocalcemia occur early, and become profound within 24–48 h of admission.

Clinical course

The rapidity with which shock and multi-organ failure can progress is impressive, and many patients may die within 24–48 h of hospitalization.[16] Shock was apparent at the time of admission or within 4–8 h in virtually all patients. In only 10% of patients did systolic blood pressure became normal 4–8 h after the administration of antibiotics, albumin, and electrolyte solutions containing salts or dopamine; in all other patients shock persisted. Interestingly, renal dysfunction preceded shock in many cases and was apparent on admission in most patients. Renal failure progressed or persisted in all patients for 48–72 h, and several patients required dialysis for 10–20 days.[16] In patients who survived, serum creatinine values returned to normal within 4–6 weeks. Acute respiratory distress syndrome (ARDS) occurred in 55% of patients and generally developed after the onset of hypotension.[16] The severity of ARDS was such that supplemental oxygen, intubation, and mechanical

ventilation was necessary in 90% who developed this syndrome.[16] Mortality rates have varied from 30% to 70%;[16,37,51] however, morbidity is also high; 13 of 20 patients in one series underwent major surgical procedures, which included fasciotomy, surgical debridement, exploratory laparotomy, intraocular aspiration, amputation, or hysterectomy.[16]

Characteristics of clinical isolates of group A streptococci

Many different characteristics have been associated with StrepTSS; however, M-types 1, 3, 6, 12, and 28 account for the majority of isolates.[8,16] Recently, 80% of strains in Sweden from all types of group A streptococcal infection have been M-type 1.[36] Pyrogenic exotoxin A (SPEA) and/or B (SPEB) were found in the majority of cases of severe infection. In the United States, SPEA is most frequently associated with these infections,[8,16,44,52–54] while in Sweden and the United Kingdom, SPEB has been most common.[51,55] Recently, streptococcal superantigen (SSA), a novel pyrogenic exotoxin, has been isolated from an M-3 strain, albeit in small concentrations.[56] In addition, mitogenic factor (MF) has been demonstrated in many different M-types of group A streptococcus.[57,58]

Pathogenic mechanisms

The role of adherence and penetration of pharyngeal epithelial cells has been the subject of intense research recently to determine which surface components of the group A streptococcus are involved, and to determine if penetration of such cells correlates with the ability of a strain to cause invasive infection. Lapenta et al.[59] demonstrated that M-1 strains were efficient at adherence and penetration of respiratory epithelial cells. Dombeck et al.[60] then demonstrated that a clonal variant of M-1 group A streptococcus linked to invasive infections (designated M1inv+) demonstrated high frequency internalization of cultured HeLa cells, and that this was dependent upon M-protein. In contrast, others have demonstrated that M-1 strains adhered to and invaded epithelial cells less efficiently than other M-types.[61,62] In addition, Molinari and Chhatwal[61] have demonstrated that, regardless of M-type, strains isolated from invasive cases (blood isolates) exhibited poorer attachment and internalization of epithelial cells than strains isolated from throat or skin.

Kawabata et al.[63] demonstrated that hyaluronic acid capsule decreased the invasion efficiency. Tsai et al.[64] investigated the role of SPEB in the invasion of respiratory epithelial cells (A-549) and found that SPEB knockout strains demonstrated reduced ability to invade cells, and that exogenous addition of SPEB increased invasion by the SPEB knockout mutants. In other studies, SPEB knockout mutants did not demonstrate reduced invasion of epithelial cells.[65] This discrepancy could, in part, be explained by the recent work of Woischnik, Buttaro, and Podbielski,[66] who demonstrated that inactivation of the SPEB structural gene (*speB*) decreased hyaluronic acid capsule production, but did not affect other virulence factors. These findings demonstrate that interpretation of data from experiments using isogenic mutants may not always be straightforward.

Talay et al.[67] and Hanski et al.[68] demonstrated that streptococcal fibronectin binding protein I (SfbI) mediated the binding of group A streptococcus to epithelial cells in the presence of fibronectin. Subsequently, Okada et al.,[69] using strains lacking the *prtF1* gene, have conclusively demonstrated that protein F, a fibronectin binding protein (SfbI), mediates the attachment of group A streptococcus to HeLa cells. Molinari and Chhatwal[61] demonstrated that the distribution of SfbI was lowest among blood isolates (27%) and highest (95%) among skin and throat isolates. These authors concluded that reduced SfbI expression was responsible for the reduced invasiveness of blood isolates.

As described under the discussion on bacterial adherence and penetration, M-protein may serve as a ligand in specific strains of group A streptococcus. Recent studies by Jadoun et al.[70] suggest that both M-protein (M-6) and SfbI may participate in the adherence and invasion of epithelial cells. This is supported by the observation that type-specific sIgA and IgG antibody prevented the adherence and invasion, respectively, of human pharyngeal cells challenged with M-6 strains.[71]

In summary, the adherence and penetration of epithelial cells by group A streptococcus is a very complex process, and it is dependent upon the cell type, state of differentiation (e.g., keratinocyte), strain of group A streptococcus, the immune status of the host, and the inflammatory milieu of the host environment. The importance of these latter points is substantiated by the observation that tumor necrosis factor (TNF) and interleukin-1 (IL-1) reduce the adherence of group A streptococcus to keratinocytes,[72] and by the demonstration that SfbI-vaccinated animals showed 80% and 90% protection against homologous and heterologous intranasal challenge with viable group A streptococcus.[73] Finally, SfbI and SfbII were found in 64% and 36% of strains in the Northern Territory in Australia, and high levels of IgG antibody were found in the sera of 80 subjects with defined streptococcal infections.[74]

Mechanisms of shock

Pyrogenic exotoxins induce fever in humans and animals, and also participate in shock by lowering the threshold to exogenous endotoxin.[17] Streptococcal pyrogenic exotoxins A and B induce human mononuclear cells to synthesize not only TNFα[75] but also IL-1β[76] and IL-6,[76–78] suggesting that TNF could mediate the fever, shock, and organ failure observed in patients with StrepTSS.[16] Pyrogenic exotoxin C has been associated with mild cases of scarlet fever in the United States (author's observations) and in England.[79] The roles of newly described pyrogenic exotoxins, such as SSA[56] and MF,[58] in the pathogenesis of StrepTSS have not been elucidated.

There is strong evidence suggesting that SPEA, SPEB, and SPEC, as well as a number of staphylococcal toxins (TSST-1, and staphylococcal enterotoxins A, B, and C) act as superantigens and stimulate T-cell responses through their ability to bind to both the class II MHC complex of

antigen-presenting cells and the V_β region of the T-cell receptor.[80] The net effect is induction of T-cell proliferation (via an IL-2 mechanism), with the concomitant production of cytokines (e.g., IL-1, TNFα, TNFβ, IL-6, and interferon [IFN]γ) that mediate shock and tissue injury. Recently, Hackett and Stevens[81] demonstrated that SPEA induced both TNFα and TNFβ from mixed cultures of monocytes and lymphocytes, supporting the role of lymphokines (TNFβ) in shock associated with strains producing SPEA. Kotb et al.[82] have shown that a digest of M-protein type 6 can also stimulate T-cell responses by this mechanism. Interestingly, quantitation of such Vβ T-cell subsets in patients with acute StrepTSS demonstrated deletion rather than expansion, suggesting that perhaps the lifespan of the expanded subset was shortened by a process of apoptosis.[83] In addition, the subsets deleted were not specific for SPEA, SPEB, SPEC, or MF, suggesting that perhaps an as yet undefined superantigen may play a role in StrepTSS.[83]

Cytokine production by less exotic mechanisms may also contribute to the genesis of shock and organ failure. Peptidoglycan, lipoteichoic acid,[84] and killed organisms[85,86] are capable of inducing TNFα production by mononuclear cells in vitro.[17,86,87] Exotoxins such as SLO are also potent inducers of TNFα and IL-1β. SPEB, a proteinase precursor, has the ability to cleave pre-IL-1β to release preformed IL-1β.[88] Finally, SLO and SPEA together have additive effects in the induction of IL-1β by human mononuclear cells.[81] Whatever the mechanisms, the induction of cytokines in vivo is likely the cause of shock, and SLO, SPEA, SPEB, and SPEC, as well as cell wall components, etc., are potent inducers of TNF and IL-1.[9] Finally, a cysteine protease formed from the cleavage of SPEB may play an important role in pathogenesis by the release of bradykinin from endogenous kininogen and by activating metalloproteases involved in coagulation.[89]

The mere presence of virulence factors, such as M-protein or pyrogenic exotoxins, may be less important in StrepTSS than the dynamics of their production in vivo. For example, Chaussee et al.[9] have demonstrated that, among strains from patients with necrotizing fasciitis and StrepTSS, approximately 40% and around 75% produced SPEA or SPEB, respectively. In addition, the quantity of SPEA, but not of SPEB, was higher for strains from StrepTSS patients compared to with patients with findings in non-invasive disease.[9] Recently, Cleary et al.[90] have proposed a regulon in group A streptococcus that controls the expression of a group of virulence genes coding for virulence factors such as M-protein and C5a-peptidase. Using DNA fingerprinting, Cleary et al.[91] showed differences in M-1 strains isolated from patients with invasive disease compared with M-1 strains from patients with non-invasive group A streptococcal infections. Such strains of group A streptococci could acquire genetic information coding for SPEA via a specific bacteriophage. Multi-locus enzyme electrophoresis demonstrates two patterns, which correspond to M-1 and M-3 type organisms that produce pyrogenic exotoxin A, a finding that fits epidemiologic studies implicating these strains in invasive group A streptococcal infections[54] in the United States.

Numerous studies have demonstrated that SPEA potentiates the action of lipopolysaccharide (LPS) in inducing shock.[92] Interestingly, this effect is largely the result of T-cell activation, and the "potentiating activity" can be passively transferred to rabbits by infusing supernatants from SPEA-stimulated lymphocytes,[93] and is likely mediated by IFN-γ and TNFβ. In contrast, the timing of pre-treatment with SPEA is crucial, because, as shown by Stevens et al.,[94] the pretreatment of mice with SPEA 96 h prior to LPS challenge resulted in protection from LPS-induced shock, probably due to the production of immunosuppressive cytokines such as IL-10. Finally, Sriskandan et al.[95] have demonstrated that animals immunized with recombinant SPEA have a higher mortality when challenged with a SPEA-producing strain of group A streptococcus than animals that are sham immunized.[95] Further Sriskandan et al.[96] have demonstrated that animals challenged with a SPEA knockout strain of group A streptococcus had greater mortality and greater tissue destruction than animals challenged with the wild-type parent strain.

Thus, the roles of the pyrogenic exotoxins in the pathogenesis of severe group A streptococcal infections are complex and remain to be fully elucidated. However, the importance of cytokine induction in group A streptococcal infections cannot be denied. Hackett et al.[85] have shown that cell wall components from killed invasive and non-invasive strains of group A streptococci elicit significant TNFα production by mononuclear cells, exceeding that elicited by SPEA, SPEB, or SLO.[85] Further, viable SLO-deficient, but not viable wild-type, group A streptococci stimulated high levels of TNFα, suggesting that SLO suppresses the induction of this cytokine, perhaps, in part, because of its cytotoxic effects on host cells. Cytokine induction by cell wall components of the group A streptococcus has also been demonstrated by Muller-Alouf et al.[86]

An important role for TNFα in the shock associated with severe group A streptococcal infections is also suggested by the remarkable efficacy of a neutralizing monoclonal antibody against TNFα in an experimental streptococcal bacteremia model in non-human primates.[97] The survival of animals was increased by 50%, and markers of organ failure, such as serum creatinine and lactate, were also significantly improved.[97]

Other mechanisms of shock and organ failure

Recently, Chatellier and Kotb[98] demonstrated that the CG rich motifs of *emm1* functioned as superantigens and induced T-cell proliferation. Sriskandan et al.[99] have also demonstrated that inducible nitric oxide (iNOS) may play a role in the shock associated with a group A streptococcal necrotizing soft-tissue infection. In that model, N-monomethyl-L-arginine (L-NMMA) administration reduced nitrate levels in serum, but did not reduce mortality.[99]

The role of streptokinase in invasive group A streptococcal infections has not been extensively studied; however, recent studies demonstrate that certain alleles of streptokinase (ska2) have three energetic folding units which change

the tertiary structure of ska into a more lipophilic molecule with high affinity for glomeruli. Only ska2 isogenic mutants are capable of causing kidney lesions with polymorphonuclear lencocyte (PMNL) influx into the glomeruli, as well as deposition of C3.[100]

Streptolysin S (SLS) has been known for a long time to be a potent cytotoxin for many different cell types, although it has only recently been implicated as a significant virulence factor. Betschel et al.[101] have demonstrated that insertional activation of the gene controlling SLS resulted in reduced virulence in a mouse model. Recently, Nizet et al.[102] have demonstrated that SLS production is controlled by a contiguous nine-gene locus, sagA to sagI, and that the SLS gene product likely has a structure similar to the bacteriocin family of microbial toxins.

Treatment and experimental therapeutics

Antibiotic therapy – importance of the mechanism of action

Streptococcus pyogenes remains exquisitely susceptible to β-lactam antibiotics, and penicillin has excellent efficacy in the treatment of erysipelas, impetigo, and cellulitis, as well as in the prevention of acute rheumatic fever. Nonetheless, clinical failures of penicillin treatment of streptococcal infection do occur, and penicillin fails to eradicate bacteria from the pharynx of patients with documented streptococcal pharyngitis in 5%–20% of patients.[103–105] Finally, more aggressive group A streptococcal infections (such as necrotizing fasciitis, empyema, burn wound sepsis, subcutaneous gangrene, and myositis) respond less well to penicillin and continue to be associated with high mortality and extensive morbidity.[4,16,17,34,55,106,107] For example, a recent report of 25 cases of streptococcal myositis reported an overall mortality of 85% despite penicillin therapy.[34]

Studies in experimental infection have demonstrated that penicillin fails when large numbers of organisms are present.[42,43] For example, in a mouse model of myositis caused by *S. pyogenes*, penicillin was ineffective when treatment was delayed for 2 h or more after initiation of infection.[43] Mice receiving clindamycin, however, had survival rates of 100%, 100%, 80%, and 70% when treatment was delayed 0, 2, 6, and 16.5 h, respectively.[43,108] Eagle[42] suggested that penicillin failed in this type of infection because of the "physiologic state of the organism." This phenomenon has recently been attributed to inoculum effects, both in vitro and in vivo.[109,110] It has also been observed that penicillin and other β-lactam antibiotics are most efficacious against rapidly growing bacteria. Early in the stages of infection, or in mild infections, organisms grow rapidly in vivo but are present in rather small numbers. With delays in treatment, higher concentrations of group A streptococci accumulate, and growth begins to slow to a stationary phase. That high concentration of *S. pyogenes* accumulate in deep-seated infection is supported by the data from Eagle.[42]

Why should penicillin lose its efficacy when large numbers of group A streptococci are present or when they are making the transition from logarithmic to stationary growth? Because penicillin mediates its antibacterial action against group A streptococci by intimately interacting with the expressed penicillin-binding proteins (PBPs), we compared the PBP patterns from membrane proteins of group A streptococci isolated from different stages of growth, i.e., mid-log-phase and stationary-phase. The binding of radiolabeled penicillin by all PBPs was decreased in stationary cells. In fact, PBPs 1 and 4 were undetectable at 36 h.[109] Thus, the loss of certain PBPs during stationary-phase growth in vitro may be responsible for the inoculum effect observed in vivo, and may account for the failure of penicillin in both experimental and human cases of severe streptococcal infection.

The greater efficacy of clindamycin in severe group A streptococcal infections is due to many factors. First, its efficacy is not affected by inoculum size or stage of growth.[109,111] Secondly, clindamycin suppresses bacterial toxin synthesis.[112,113] Third, clindamycin facilitates the phagocytosis of *S. pyogenes* by inhibiting M-protein synthesis.[113] Fourth, clindamycin suppresses the synthesis of PBPs which, in addition to being targets for penicillin, are also enzymes involved in cell wall synthesis and degradation.[111] Fifth, clindamycin has a longer post-antibiotic effect than β-lactams such as penicillin. Lastly, we have recently shown that clindamycin suppresses LPS-induced monocyte synthesis of TNFα.[114] Thus, clindamycin's efficacy may also be related to its ability to modulate the immune response to group A streptococcal infection. Interestingly, in a recent retrospective analysis of StrepTSS cases, Zimbelman et al.[115] demonstrated significantly improved survival in patients receiving clindamycin compared with survival in those treated with β-lactam antibiotics.

Other treatment measures

Prompt and aggressive exploration and debridement of suspected deep-seated *S. pyogenes* infection is crucial to limit complications, as well as to prevent extension to vital areas that may be impossible to debride (i.e., the head and neck, thorax, or abdomen). Because definite cutaneous evidence of necrotizing fasciitis and myositis may not appear until late in the course, the index of suspicion should be high in patients with fever, excruciating pain, and systemic toxicity. Radiographs and CT and MRI scans in patients with StrepTSS show soft-tissue swelling and an absence of gas, thus providing clues to the site of the deep infection. Prompt surgical exploration through a small incision, with visualization of muscle and fascia, and timely Gram stain of surgically obtained material provide the earliest and most definitive etiologic diagnosis.[40]

Because of intractable hypotension and diffuse capillary leak, massive amounts of intravenous fluids (10–20 l/day) are often necessary, and about 10% of patients have significant clinical improvement. Pressors such as dopamine are used frequently, although no controlled trials have been performed in StrepTSS. In patients with intractable

hypotension, vasoconstrictors such as epinephrine have been used, but frequently, symmetrical gangrene of digits results (author's unpublished observations). Loss of all digits on both hands and feet, or loss of both arms and legs has occurred in this setting. In these cases it is difficult to determine whether the symmetrical gangrene is caused by the pressors or by the infection, or by both.

Neutralization of circulating toxins would be a desirable therapeutic modality, yet appropriate antibodies are not commercially available in the United States or Europe. Case reports,[116,117] and one non-randomized clinical trial,[118] report that commercial intravenous gamma globulin (IVIG) has been useful for treating StrepTSS. A double-blind study using IVIG is currently underway in northern Europe. Because cytokines are important mediators of shock in StrepTSS, strategies to inhibit or neutralize their effects may provide useful treatments. Recently, a monoclonal antibody against TNFα showed promising efficacy in a baboon model of StrepTSS.[97] Finally, anecdotal reports suggest that hyperbaric oxygen may be helpful; however, no controlled studies are underway, nor is it clear if this treatment is useful.

Prevention

Vaccines

Currently, several group A streptococcal virulence factors are being investigated as potential vaccine candidates. These include: group A polysaccharide, C5a peptidase, SPEB, SPEA, the conserved segment of the M-protein molecule, and a cassette of peptides representing the hypervariable regions of M-protein from strains commonly encountered. At the present time, none have been studied in humans.

Prophylaxis and the risk of secondary cases of StrepTSS

The risk of secondary cases of StrepTSS must be low, based on the low prevalence of this disease despite a high prevalence of "virulent strains of group A streptococci" in the population at large.[2] Yet, clusters of group A streptococcal invasive infections have been described in nursing homes[49,50,119] in healthcare workers,[46,120] and among family members.[46,119] Finally, patients may acquire group A streptococci from hospital personnel, and this was best demonstrated historically by Semmelweis, in Vienna, and Holmes, in the United States, although even in modern times such transmission is well documented.[46,120,121] Epidemiologic investigation of clusters of cases is important, and treatment of contacts may be necessary despite the low risk of secondary invasive infection.[122] Primary care physicians must consider the extent of exposure, the type of exposure, and the risk factors of the contact. For example, a contact of a case of StrepTSS with risk factors such as chicken pox, leukemia, burns, or recent surgery, or any open skin lesion, should receive prophylaxis with penicillin, erythromycin, or clindamycin.[122]

Acknowledgments This material is based upon work supported by the Office of Research and Development, Medical Research Service, Department of Veterans Affairs.

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